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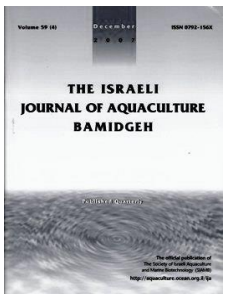

Effects of Three Feeding Modes on the Gonad Development and Nutritional Quality of the Female Crab *Portunus trituberculatus*

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Keywords: three feeding modes; *Portunus trituberculatus;* gonad development; nutritional quality

Abstract

Portunus trituberculatus is an important aquaculture species in China. Presently, three main feeding models are used for *P. trituberculatus*: complete feeding with commercial formulated feed (M1), mixed feeding with commercial formulated feed and trash fish (M2) and complete feeding with trash fish (M3). This study assessed the gonad development and nutritional quality of female *P. trituberculatus* under the three feeding models and provides a scientific basis for optimizing the feeding models of *P. trituberculatus* in the future. Overall, this study concluded that hypoplasia of gonads, and the biochemical, fatty acid, and amino acid composition in the gonads, hepatopancreas, and muscle of *P. trituberculatus* fed with the commercial formulated diet were poor. The mixed feeding with the commercial formulated diet and trash fish was an ideal feeding mode. This mode not only alleviates the dependence of the *P. trituberculatus* breeding industry on miscellaneous fish resources but also ensures the excellent quality of *P. trituberculatus*.

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Introduction

Portunus trituberculatus is an economically important marine crab with delicious, highly nutritious, meat. Since the breakthrough of artificial breeding technology in the 1990s, the scale of *P. trituberculatus* breeding has gradually expanded, and this crab has become one of the main species to be cultured and bred in the coastal areas of China. In 2017, the national aquaculture output of *P. trituberculatus* was 120,000 tons (China Fisheries Statistics Yearbook, 2018).

The conventional feeding mode of *P. trituberculatus* has always been the wild trash fish model; however, problems such as unstable food source, uncontrollable quality, pathogen transmittal, and easily poluted water quality have seriously affected the sustainable development of the *P. trituberculatus* farming industry (Zhao & Han,2017). Consequently, the nutritional needs of *P. trituberculatus* and development of formulated feed have attracted much attention (Jin et al., 2013, Huo et al., 2014, 2017). Following years of efforts, commercial formulated feed for *P. trituberculatus* entered the Chinese market and is gradually being popularized and applied throughout the country. Due to the different acceptance of formulated feeds by *P. trituberculatus* aquaculture practitioners, three main feeding modes exist at present. The first mode is the complete feeding of formulated feeds, the second mode is the mixed feeding of formulated feeds and trash fish, and the third mode is the complete feeding of trash fish.

Feeding modes (including the types and composition of feed) not only affect the survival rate, growth rate, and disease resistance of aquaculture animals but also directly affect the nutritional quality of the aquaculture products (Wu et al., 2014). Hence, the nutritional quality of aquaculture animals has become one of the crucial criteria for evaluating the quality of feeding models (Li et al., 2017). Until now, many reports have existed about the effects of different diet models and the replacement of fresh diets with compound diets on the nutritional quality of aquatic animals (Jiang et al., 2012, Shao et al., 2013, 2014; Shi et al, 2014; Shi et al., 2014).

The hepatopancreas, gonads, and muscles are the primary edible parts of *P. trituberculatus.* This study compared the biological parameters of the hepatopancreas index, gonad index, and meat yield of female *P. trituberculatus* reared with three feeding models by determining and comparing the nutritional, fatty acid, and amino acid composition in the hepatopancreas, gonad, and muscle to evaluate the nutritional quality of female *P. trituberculatus* under the three feeding models, and provides a theoretical basis for optimizing the feeding mode of *P. trituberculatus* in the future.

Materials and methods

Source of female crabs

The experiment was conducted on July 1, 2017, at the *P. trituberculatus* breeding base of Zhejiang Marine Aquaculture Research Institute. The 0.15 hectare pond was divided into 9 enclosures each of which was approximately 200 square metres with plastic mesh. Three feeding modes were established: the commercial formulated feed group (M1), mixed commercial formulation and trash fish as feed group (M2), and trash fish only as feed group (M3), with three enclosures in each group. A total of 450 healthy female juvenile crabs, (average weight of 4.50±0.52 g), were cultured in each enclosure beginning on July 1. From July to November, crabs were fed daily at 5 pm, the feeding response was observed at 6 am the next day, the amounts fed were adjusted in a timely manner, and feeding was terminated after November 20, when the water temperature was low. The culture water was changed 1-2 times a week according to tidal changes, and 1/3 of the water volume was changed each time. The water level was deepened during high temperature periods and was supplied with continuous oxygenation throughout the day (DO > 4 mg/L). The water quality was tested weekly. pH was maintained at 7.0-8.0, nitrite concentration < 0.15 mg/L, and ammonia concentration < 0.5 mg/L.

Sample collection

Nine crabs with complete limbs were chosen from each feed group at random in late January, and after removing water from the surface of the crabs with an absorbent cloth, the crabs were weighed (± 0.01 g). The whole hepatopancreas, ovaries, and muscles were dissected, isolated from each crab and weighed with an electronic balance (± 0.001 g). The hepatosomatic index (HSI), gonadosomatic index (GSI), meat yield (MY), as well as the total edible yield (TEY), were determined as follows:

HSI(%)=WH/W×100%

GSI(%)=WG/W×100%

MY (%)= WM/W×100%

TEY(%)= HSI(%)+ GSI(%)+ MY(%)

Where WH = hepatopancreas weight, WG = Gonad weight, WM = Muscle weight, and W = Body weight. All samples were stored in a refrigerator at -40°C, for subsequent determination of the biochemical composition.

Biochemical analysis

Nutritional composition analysis

The components of the muscles and gonads were measured according to the AOAC (1995). The moisture content was determined by extracting water in a lyophilizer (LL1500: Thermo, USA) for 24 hours by a freeze-drying method; crude protein was calculated by the Kjeldahl method (K438/K355, BUCHI, Flawil, Switzerland), and the Soxhlet method (E-816, BUCHI, Flawil, Switzerland) was used for the determination of crude fat. Ash determination followed tissue carbonization in an electric furnace for 3 hours and burning in a muffle furnace at 550°C for 12 hours.

Fatty acid composition analysis

The fatty acid content was determined according to Wu (2010). Fat was extracted from the muscles, hepatopancreas, and gonads of crabs with a chloroform-methanol solution (2:1, v/v), and fatty acid methyl ester (FAME) was acquired by esterification of 14% boron trifluoride-methanol (V:V). Afterwards, the FAME was subjected to analysis by gas chromatography (Agilent 6890). The type of capillary column was Omegawax 320 (30.0 m×0.32 mm, USA), and the temperature of the sample injection and sampling port was 260°C, with the initial temperature of 60°C being raised gradually to 260°C by programmed heating until all fatty acids were isolated. The air flow rate was 300 mL/min, the hydrogen flow rate was 30 mL/min, the carrier gas was 25 mL/min helium, the shunt ratio was 1:50, and the pressure was 60 kPa. The fat composition and fatty acid content were calculated using the area percentage method.

Amino acid analysis

The total amino acid contents of the muscle and gonad were determined according to Chen et al (2007). Initially, a freeze-dried sample of approximately 0.1 g was placed in 6 mol/L hydrochloric acid; then, the sample was acidified in an oven at 110°C for 24 hours. After acidification, the supernatant was centrifuged to 50 mL with distilled water, and thereafter, the supernatant was filtered through a filter with a diameter of 0.45 μm. The supernatant of 1 mL was steamed repeatedly (2-3 times) at 50°C to remove the hydrochloric acid, and subsequently, it was dissolved in 0.02 mol/L hydrochloric acid. Ultimately the solution of 1 mL was used for the amino acid analysis in Hitachi 835-50 amino acid automatic analyser.

Data analysis

All data were expressed as the Mean ± Standard Error (Mean ± SE). SPSS 17.0 software was applied to analyse the experimental data, and the Levene method was used to test the homogeneity of the variance. When the data did not satisfy the homogeneous variance, the percentage data were processed by arcsine or square root. Independent samples were tested by the t-test to check for differences in each index among the different groups; $P < 0.05$ indicated a significant difference, and $P < 0.01$ indicated an extremely significant difference.

Results

Effects of three feeding modes on the biological index and total edible yield of female P. trituberculatus

Table 1 shows that the biological index and total edible yield of female *P. trituberculatus* were affected by the three feeding modes. Among the three modes, M1 had the lowest HSI, GSI, and MY, whereas the HSI, GSI, and MY were greater in M3, of which the GSI of M1 was only 2.99%, remarkably lower than that of the M3 (5.70%). Moreover, the HSI, GSI and MY in M2 were between M1 and M3, and no notable difference was observed with the M3 ($P > 0.05$). The discrepancy in the biological index indicates that the TEY of M1 is lower than that of M3 ($P < 0.05$), whereas the TEY of M2 is slightly lower than that of the M3.

Table 1. Effects of three feeding modes on the biological index and total edible rate of female *P. trituberculatus*

Index	*M1*	*M2*	*M3*
HSI (%)	5.89±1.24[a]	6.50±1.03[a]	7.59±0.97[a]
GSI (%)	2.99±0.68[a]	4.64±0.86[ab]	5.70±0.89[b]
MY (%)	27.38±2.17[a]	30.04±2.87[a]	30.47±1.33[a]
TEY (%)	36.26±3.08[a]	41.18±4.07[ab]	43.76±2.60[b]

HIS = hepatosomatic index GSI= gonadosomatic index, MY = meat yield, and TEY = total edible yield

Effects of three feeding modes on conventional biochemical composition of edible parts of female P. trituberculatus

The biochemical composition of the hepatopancreas, gonads, and muscle of female *P. trituberculatus* in the three feeding modes is presented in Table 2. For the hepatopancreas, M1 had obvious advantages over M3 in moisture content, whereas the fat content was distinctly lower in M3 ($P < 0.05$). The moisture and fat content in M2 were between those contents observed in M1 and M3, and no distinct difference was observed in the protein and ash content of M1, M2 and M3 ($P > 0.05$). The gonads showed a difference in the moisture and protein content among the three feeding modes. The moisture content followed the order of M1 > M2 > M3; moreover, M1 notably exceeded M3, whereas the protein content was just the opposite, with the protein content of M1 and M2 being lower than that of M3, and M1 was notably different from M3. In the muscles, moisture, protein, fat and ash content of the three feeding modes were similar, and no noticeable differences existed among the three groups ($P > 0.05$).

Table 2. Effect of three feeding modes on conventional biochemical composition of the hepatopancreas, gonads and muscles of female *P. trituberculatus* (g/100 g of wet weight).

Tissue	*M1*	*M2*	*M3*
Hepatopancreas			
Moisture	64.43±4.30[b]	59.19±4.78[ab]	55.28±4.18[a]
Protein	9.24±0.74[a]	9.05±0.61[a]	9.54±0.91[a]
Fat	21.56±2.52[a]	25.87±3.11[ab]	30.38±3.21[b]
Ash	1.24±0.37[a]	1.61±0.39[a]	1.39±0.22[a]
Gonad			
Moisture	62.93±4.32[a]	60.07±5.64[a]	57.53±5.93[a]
Protein	23.34±2.38[a]	26.81±0.64[ab]	28.01±1.07[b]
Fat	7.57±0.75[a]	8.12±1.76[a]	8.18±1.07[a]
Ash	2.55±0.06[b]	2.36±0.41[ab]	2.18±0.20[a]
Muscle			
Moisture	80.02±4.60[a]	80.23±3.60[a]	78.83±1.56[a]
Protein	13.28±3.94[a]	14.91±2.37[a]	16.41±1.29[a]
Fat	2.01 ±0.25[a]	1.75±0.28[a]	1.83±0.31[a]
Ash	1.49±0.18[a]	1.50±0.07[a]	1.43±0.17[a]

Effects of three feeding modes on fatty acid composition of the edible part of female P. trituberculatus.

Table 3 illustrates that the fatty acid composition of the hepatopancreas of female *P. trituberculatus* was basically the same among the three feeding modes. The saturated fatty acids were mainly C16:0 and C18:0; the monounsaturated fatty acids were mostly C18:1n9, C16:1n7 and C22:6n3; and C20:5n3 dominated the polyunsaturated fatty acids. The saturated fatty acid content and the ΣSFA in the three feeding modes were similar, and the content of the monounsaturated fatty acids in M1 and M2 were somewhat stronger than those in M3 such that the contents of ΣMUFA in M1 and M2 strikingly exceeded those in M3 ($P < 0.05$).

The C18:2n6 and C18:3n3 content of M1 predominated in all the modes at the same time, and the content of C22:6n3 in M3 had an advantage over that in M1. The ΣHUFA, Σn-3 PUFA/ Σn-6 PUFA and DHA/EPA of the three feeding modes were greatest in M3, followed by M2 and least in M1; meanwhile, the DHA/EPA in M1 was lower than in M2 and M3 ($P < 0.05$), with no distinct statistical difference observed between M2 and M3.

Table 3. Effect of three feeding modes on fatty acid composition of hepatopancreas of female *P. trituberculatus* (% of total FA)

Fatty acids	*M1*	*M2*	*M3*
C14:0	0.81±0.18^{a}	2.43±0.05^{b}	2.12±0.23^{b}
C15:0	0.30±0.06^{a}	0.46±0.03^{b}	0.44±0.00^{b}
C16:0	17.75±0.85^{a}	17.93±0.54^{a}	17.36±0.26^{a}
C17:0	0.80±0.20^{a}	1.18±0.02^{b}	1.19±0.06^{b}
C18:0	5.77±0.79^{a}	4.69±0.49^{a}	5.01±0.42^{a}
C20:0	0.43±0.06^{a}	0.51±0.07^{a}	0.49±0.06^{a}
C22:0	0.34±0.12^{a}	0.34±0.05^{a}	0.32±0.03^{a}
ΣSFA	26.54±1.3^{a}	27.59±1.10^{a}	27.30±0.26^{a}
C16:1n7	8.88±0.68^{a}	9.15±0.74^{a}	7.32±1.46^{a}
C17:1n7	0.81±0.32^{a}	1.09±0.18^{a}	1.07±0.17^{a}
C18:1n9	38.00±1.24^{a}	36.58±0.69^{a}	35.25±1.57^{a}
C20:1n9	1.84±0.25^{a}	1.92±0.62^{a}	1.72±0.93^{a}
C22:1n9	0.87±0.13^{b}	0.53±0.05^{a}	0.51±0.11^{a}
C24:1n9	0.56±0.20^{a}	0.84±0.07^{a}	0.77±0.08^{a}
ΣMUFA	50.86±1.33^{b}	50.11±0.73^{b}	46.64±0.85^{a}
C18:2n6	2.82±0.26^{b}	1.80±0.15^{a}	1.83±0.05^{a}
C18:3n3	3.07±0.54^{a}	1.82±1.23^{a}	2.27±1.38^{a}
C20:2n6	0.50±0.07^{a}	0.84±0.21^{a}	1.04±0.05^{b}
C20:4n6	1.74±0.68^{a}	1.29±0.11^{a}	1.56±0.31^{a}
C20:5n3	3.42±0.27^{a}	3.24±0.31^{a}	3.67±0.27^{a}
C22:5n3	1.43±0.42^{a}	1.53±0.20^{a}	1.80±0.12^{a}
C22:6n3	8.62±0.89^{a}	11.01±1.47^{ab}	13.29±0.7^{b}
ΣPUFA	21.90±1.10^{a}	21.68±0.44^{a}	25.70±1.18^{b}
Σn-3PUFA	16.54±0.61^{a}	17.60±0.76^{a}	21.03±0.97^{b}
Σn-6PUFA	5.35±1.23^{a}	4.08±0.31^{a}	4.67±0.22^{a}
n3/n6	3.26±0.72^{a}	4.38±0.52^{a}	4.50±0.05^{a}
ΣHUFA	15.51±1.16^{a}	17.22±2.03^{ab}	20.57±1.18^{b}
DHA/EPA	2.55±0.39^{a}	3.37±0.13^{b}	3.62±0.14^{b}

Note: fatty acids representing more than 0.3% of the total fatty acids are shown in the table.

According to Table 4, the fatty acid composition of female gonads of *P. trituberculatus* conclusively resembled that of the hepatopancreas; however, C22:6n3 and C20:5n3 in gonads were predominant compared to those in the hepatopancreas. The contents of the various saturated fatty acids and ΣSFA were roughly homogeneous among the three feeding modes. Monounsaturated fatty acid content in M1 was lower than that observed in M2 and M3, of which C16:1n7 and C17:1n7 were the most obvious, and ΣMUFA was lower in M2 compared to M3 and even lower in M1. Inversely, most polyunsaturated fatty acids were higher in M1 than in M2 and M3. Nevertheless, ΣPUFA and ΣHUFA were approximately uniform among the three feeding modes ($P > 0.05$). Σn-3PUFA/Σn-6PUFA and DHA/EPA in M1 were significantly lower than in M2 and M3 ($P < 0.05$); however, the contents and ratios of the various polyunsaturated fatty acids in M2 and M3 were very similar.

Table 4. Effect of three feeding modes on fatty acid composition of gonads of female *P. trituberculatus* (% of total FA)

Fatty acids	*M1*	*M2*	*M3*
C14:0	1.35±0.34[a]	1.98±0.13[a]	1.93±0.09[a]
C16:0	17.71±1.75[a]	19.63±0.81[a]	19.60±1.45[a]
C17:0	0.34±0.00[b]	0.29±0.02[a]	0.34±0.03[ab]
C18:0	6.05±0.15[a]	6.09±0.29[a]	6.16±0.25[a]
C24:0	0.38±0.09[a]	0.44±0.01[a]	0.49±0.08[a]
ΣSFA	26.11±1.70[a]	28.77±1.24[a]	28.81±1.71[a]
C16:1n7	3.28±1.22[a]	7.46±0.70[b]	6.17±0.59[b]
C17:1n7	0.45±0.07[a]	0.71±0.01[b]	0.85±0.09[b]
C18:1n9	28.74±0.99[a]	30.28±0.63[a]	28.88±0.44[a]
C22:1n9	0.37±0.09[a]	0.30±0.02[a]	0.34±0.04[a]
ΣMUFA	33.35±0.90[a]	39.21±0.29[c]	36.68±1.02[b]
C18:2n6	3.69±0.88[b]	1.37±0.22[a]	1.46±0.13[a]
C18:3n3	1.29±0.22[a]	0.98±0.03[a]	1.02±0.09[a]
C20:2n6	1.48±0.24[b]	0.41±0.07[a]	0.64±0.24[a]
C20:4n6	2.33±0.69[a]	2.55±0.05[a]	2.33±0.32[a]
C20:5n3	8.95±1.33[a]	6.91±0.35[a]	7.55±0.87[a]
C22:5n3	1.24±0.27[a]	1.78±0.16[ab]	1.85±0.09[b]
C22:6n3	17.94±2.11[a]	17.98±1.07[a]	19.70±1.65[a]
ΣPUFA	37.14±3.67[a]	32.04±1.42[a]	34.61±2.28[a]
Σn-3PUFA	29.43±2.79[a]	27.66±1.23[a]	30.12±2.46[a]
Σn-6PUFA	7.71±1.13[b]	4.39±0.33[a]	4.49±0.20[a]
n3/n6	3.86±0.37[a]	6.33±0.39[b]	6.74±0.85[b]
ΣHUFA	30.68±2.99[a]	29.29±1.25[a]	31.49±2.17[a]
DHA/EPA	2.04±0.36[a]	2.61±0.21[b]	2.62±0.08[b]

Note: fatty acids representing more than 0.3% of the total fatty acids are shown in the table.

Compared with the hepatopancreas and gonads, the fatty acid composition in the muscle of female *P. trituberculatus* in the three feeding modes showed minimal differences (Table 5). When the three feeding modes were compared, the content of most of the saturated fatty acids and monounsaturated fatty acids, except for C18:1n9, were practically identical (including the SFA and MUFA contents); the C22:6n3 content in M1 far surpassed the content in M2 and M3 ($P < 0.05$), indicating a discrepancy in the polyunsaturated fatty acids. Correspondingly, the Σn-3PUFA/Σn-6PUFA and DHA /EPA in M1 were less than those in M2 and M3. Regardless of the composition of saturated fatty acid, monounsaturated fatty acid or polyunsaturated fatty acid, M2 and M3 were very similar ($P > 0.05$).

Table 5. Effect of the three feeding modes on the fatty acid composition of muscles of female *P. trituberculatus* (% of total FA)

Fatty acids	*M1*	*M2*	*M3*
C16:0	15.49±0.77[a]	17.03±0.64[a]	16.48±1.23[a]
C17:0	0.40±0.08[a]	0.58±0.32[a]	0.69±0.22[a]
C18:0	8.08±1.27[a]	6.88±0.37[a]	6.95±0.14[a]
ΣSFA	23.98±0.81[a]	24.48±0.74[a]	24.12±0.88[a]
C16:1n7	1.87±0.82[a]	5.47±0.80[b]	4.33±0.45[b]
C17:1n7	0.37±0.11[a]	0.70±0.04[a]	0.50±0.21[a]
C18:1n9	33.02±0.42[b]	30.08±1.53[a]	30.07±0.02[a]
C20:1n9	0.58±0.15[b]	0.43±0.08[ab]	0.22±0.09[a]
ΣMUFA	35.84±0.43[a]	36.67±1.37[a]	35.12±0.59[a]
C18:2n6	2.47±0.32[a]	2.13±0.74[a]	2.71±0.79[a]
C18:3n3	0.56±0.06[a]	0.45±0.07[ab]	0.33±0.02[a]
C20:2n6	1.75±0.42[b]	0.40±0.13[a]	1.06±0.49[ab]
C20:4n6	3.55±0.90[a]	3.42±0.20[a]	3.11±0.07[a]
C20:5n3	18.71±1.31[a]	16.11±0.79[a]	17.53±0.97[a]
C22:5n3	0.58±0.13[a]	0.70±0.02[a]	0.80±0.01[a]
C22:6n3	10.56±2.19[a]	15.63±1.15[b]	15.22±0.68[b]
ΣPUFA	38.18±1.55[a]	38.84±2.11[a]	40.76±1.46[a]
Σn-3PUFA	30.41±1.86[a]	32.89±1.79[a]	33.88±0.25[a]
Σn-6PUFA	7.77±0.64[a]	5.95±0.77[a]	6.88±1.21[a]
n3/n6	3.96±0.54[a]	5.62±0.77[a]	5.15±0.87[a]
ΣHUFA	33.40±2.11[a]	35.87±2.01[a]	36.65±0.21[a]
DHA/EPA	0.57±0.14[a]	0.97±0.04[b]	0.88±0.09[b]

Note: the fatty acids representing more than 0.3% of the total fatty acids are shown in the table.

Effects of three feeding modes on the amino acid composition of the edible part of female P. trituberculatus.

The content of 18 amino acids in the glands of female *P. trituberculatus* were determined for three feeding modes. Table 6 illustrates how the contents of the various amino acids in M1 were more deficient than those in M2 and M3, for example, Ile, Leu, Lys, Phe, Tyr, Thr, Val, Asp, Ser, Glu, Gly, Ala, and His, with a large difference between M1 and M3. Accordingly, the content of EAA and NEAA, along with TAA, in M1 did not exceed that observed for M2 and M3 ($P < 0.05$), whereas the EAA/TAA in M1 approached that in M2 and M3, ranging between 0.47 and 0.48. The content of EAA, NEAA, and TAA in M2 showed no major difference to that observed in M3, as they were basically the same; therefore, no difference ($P > 0.05$) existed.

Table 6. Effects of three feeding modes on the amino acid composition of gonads of female *P. trituberculatus* (mg/g of wet weight)

Amino	*M1*	*M2*	*M3*
Ile	6.15±0.05[a]	12.31±2.30[b]	11.97±1.40[b]
Leu	10.91±0.25[a]	20.55±1.93[b]	19.23±2.59[b]
Lys	8.90±0.24[a]	17.32±0.51[b]	17.30±1.45[b]
Met	3.82±0.30[a]	4.51±1.80[a]	3.95±0.96[a]
Cys	4.25±0.41 [a]	5.01±0.76 [a]	4.84±0.60 [a]
Phe	6.70±0.58[a]	11.29±1.23 [b]	11.31±1.64 [b]
Tyr	6.94±0.36[a]	12.33±1.27 [b]	12.15±1.72 [b]
Thr	8.25±0.92[a]	13.21±1.59[b]	13.36±1.05[b]
Val	9.09±0.81[a]	15.30±0.99 [b]	15.54±1.37 [b]
Trp	7.23±0.84 [a]	10.84±1.23 [a]	10.22±1.01 [a]
EAA	72.26±4.65[a]	122.67±12.18[b]	119.88±10.72[b]
Asp	11.94±1.13[a]	20.21±2.06[b]	20.32±2.07[b]
Ser	7.81±0.29[a]	13.84±2.61[b]	13.70±1.76[b]
Glu	17.85±0.47[a]	32.63±3.64[b]	32.65±2.87[b]
Gly	7.94±1.86[a]	11.36±1.58[b]	11.85±1.47[b]
Ala	7.11±0.54[a]	11.68±1.79[b]	12.21±0.51[b]
His	4.57±0.59[a]	7.28±1.52[b]	7.57±1.34[b]
Arg	12.77±2.52[a]	17.55±0.91[a]	18.69±1.37[a]
Pro	8.08±0.69[a]	15.93±2.8[a]	16.15±2.36[a]
NEAA	78.06±6.79[a]	130.48±17.83[b]	133.14±12.66[b]
TAA	150.32±11.44[a]	253.14±30.01[b]	253.02±23.38[b]
EAA/TAA	0.48±0.02[a]	0.48±0.01[a]	0.47±0.01[a]

As shown in Table 7, the amino acid composition disparity in the muscles of female *P. trituberculatus* in the three feeding models was tiny. Subsequently, the contents of the various essential amino acids in M1 surpassed slightly those in M2 and M3; likewise, the EAA contents were significantly greater than those in M3. The amounts of the various NEAA and TAA in the three feeding models were similar; EAA/TAA in M2 and M3 did not exceed the levels observed in M1 (0.43), with both showing levels of 0.39.

Table 7. Effects of three feeding modes on the amino acid composition of muscles of female *P.*

Amino	*M1*	*M2*	*M3*
Ile	7.07±0.30[a]	6.43±0.53[a]	6.11±1.10[a]
Leu	12.05±0.53[a]	10.81±0.97[a]	10.16±1.92[a]
Lys	13.23±0.51[a]	11.76±1.36[a]	10.71±0.43[a]
Met	3.17±1.00[a]	3.44±0.21[a]	2.21±0.38[a]
Cys	2.25±0.18 [a]	2.63±0.25 [a]	2.31±0.21 [a]
Phe	6.76±0.25[a]	6.12±0.28[a]	5.88±1.30[a]
Tyr	6.42±0.26[a]	5.83±0.42[a]	5.64±1.00[a]
Thr	7.17±0.28[a]	6.33±0.54[a]	6.00±1.08[a]
Val	7.28±0.33[a]	6.69±0.48[a]	6.34±1.21[a]
Trp	2.63±0.30 [a]	2.40±.017 [a]	2.55±0.29 [a]
EAA	68.02±1.94[b]	62.44±4.79[ab]	57.91±4.82[a]
Asp	12.87±0.77[a]	14.07±1.17[a]	13.36±2.42[a]
Ser	4.90±0.23[a]	5.93±0.53[a]	5.54±1.07[a]
Glu	23.65±1.9[a]	23.18±1.65[a]	21.81±1.58[a]
Gly	12.76±1.35[a]	14.16±3.20[a]	11.25±1.81[a]
Ala	10.10±1.98[a]	11.44±1.37[a]	10.48±1.52[a]
His	3.86±0.25[a]	3.53±0.17[a]	3.53±0.60[a]
Arg	13.56±1.40[a]	14.73±2.17[a]	18.03±2.31[a]
Pro	8.39±2.07[a]	8.76±0.34[a]	9.29±1.7[a]
NEAA	90.08±7.88[a]	95.81±10.26[a]	90.30±10.29[a]
TAA	158.10±9.82[a]	158.25±15.05[a]	148.21±15.11[a]
EAA/TAA	0.43±0.02[a]	0.39±0.01a	0.39±0.02[a]

Essential amino acid scores (EAAS) in the ovaries and muscles of *P. trituberculatus* were evaluated according to FAO/WHO/UNU criteria (Table 8). In the gonads, most of the amino acid scores in M1 were smaller than those in M2 and M3, especially the Ile, Leu, Lys, and Phe + Tyr, which were not above 100 and are restrictive amino acids. Amino acid contents in M2 in the gonads exceeded those in M3 only marginally. In contrast, in the muscles, M1 had high amino acid scores, which were followed by M2 and M3.

Table 8. Effects of three feeding modes on the essential amino acids score (EAAS) of ovary

	Gonads			*Muscle*		
EAA	*M1*	*M2*	*M3*	*M1*	*M2*	*M3*
Ile	94	164	153	190	154	133
Leu	71	116	104	137	110	94
Lys	66	111	106	172	136	113
Met +Cys	138	142	126	163	163	110
Phe + Tyr	93	140	133	158	127	111
Thr	104	145	140	159	125	108
Trp	282	368	332	164	136	115
Val	111	163	159	157	128	110
Mean value	120	169	157	153	125	103

Discussion

The hepatopancreas, gonads and muscles are the most important edible parts of *P. trituberculatus,* and the proportions of these three products in the body mass are a crucial index used to evaluate the quality of commercial crabs. The quality of the gonads (ovaries) of female crab, especially, are directly related to the sales price of *P. trituberculatus* (Wu et al., 2014; He et al., 2018). Previous research has shown that the use of complete substitution or partial substitution of trash fish for compounded feed in pond culture exerts an indistinct effect on the growth of the decidua in *P. trituberculatus.* In contrast, our study showed that the gonad index of female *P. trituberculatus* fed entirely with compounded feed was visibly inferior to that fed the complete trash fish diet. These results suggest that, although the compounded feed could better meet the nutritional needs of *P. trituberculatus* during the growth period for the decidua, it could not meet the nutritional needs of the crab during its later gonadal development, resulting

in gonadal dysplasia. Because the hepatopancreas index of *P. trituberculatus* in the group receiving the compounded feed group was lower than that in the trash fish group, an insufficient supply of feed nutrients during gonadal development is speculated to promote the transfer of more nutrients from the hepatopancreas to the gonads. The hepatopancreas as a source of energy for gonad development has been confirmed for *Eriocheir sinensis*, *Portunus pelagicus*, *Cancer magister* and other crabs (Allen et al., 1972; Cheng et al, 1997; Liu et al., 2014). In addition, the lower muscle percentage in the group receiving the compounded feed may be affiliated with the fact that *P. trituberculatus* also uses the nutrients in the muscles during gonadal development. In contrast, complete feeding of commercial feeds significantly affected the total edible rate of the swimming crab, whereas the mixed feeding mode of commercial feed and trash fish had less effect.

The species, germplasm, sex, stage of development, exogenous water quality conditions, feed composition, etc. affect the nutrient composition of aquatic animals (People Le Ruyet et al., 1993; Shi et al., 2014). In this study, under conditions with a consistent seedling source, water quality and environment, the effect of feeding commercial formulated feed instead of trash fish on the nutritive composition of the edible parts of *P. trituberculatus* was confirmed. The hepatopancreatic fat content of the group receiving the compounded feed and the group receiving the mixed feed was lower than that in the group receiving only trash fish, and it was more obvious in the group receiving the compounded feed. We believes that the poor fat content of the hepatopancreas caused by the replacement of trash fish with the compounded diet occurred because *P. trituberculatus* transfers more fat from the hepatopancreas to the gonads under conditions of indigent exogenous nutrition, which is exactly the reason why the fat content of the gonads of *P. trituberculatus* in the replacement group is similar. Sui et al (2010) reported that, during the gonad development of the female mitten crab, the lipid composition in the hepatopancreas decreased with the increase of the gonad fat content. The moisture in the hepatopancreas and gonads was greater in the group receiving the compounded feed, which was correlated with the nethermost fat and protein content in these two tissues. Relative to the effects observed on the hepatopancreas and gonads, the muscle biochemical components of *P. trituberculatus* were less affected by the feeding mode. From a biochemical perspective, feeding of commercial formulated feed abated the fat content in the hepatopancreas and the protein content in the gonads.

The fatty acid types of the hepatopancreas, ovary and muscle of the *P. trituberculatus* in the three feeding models were consistent, and the saturated fatty acid (SFA) was dominated by c16:0, the monounsaturated fatty acid (MUFA) was dominated by c18:1n9, and the polyunsaturated fatty acid (PUFA) was dominated by c20:5n3 and c22.6n3, indicating conservative and stable fatty acid composition of the *P. trituberculatus* in miscellaneous tissues. Wu et al. (2010) insisted that lipids in feed need to be absorbed by the hepatopancreas and then transported to other tissues; hence, the fatty acid composition of the hepatopancreas is generally affected by feed to a great extent, whereas the fatty acid composition of the muscle has a certain conservative form and is less affected by feed. A comparable mode was also found in this study; that is, the difference in fatty acids in the hepatopancreas was most conspicuous among the three feeding models and was mainly observable for high unsaturated fatty acids (HUFA). In three kinds of feeding models, the hepatopancreas DHA and Σ HUFA content in the total substitution group was far below that of the trash fish group, and the mixed feeding model was also mildly impacted. However, in *P. trituberculatus* groups fed the complete commercial feed or the mixed feed, the DHA and Σ HUFA contents (in the gonads and muscles) are relatively similar to those of the trash fish group. In contrast, the Σ n-3 pufa/Σ n-6 PUFA ratio is a good approach to for observing responses to discrete food nutrition values, with the Σ n-3/n-6 PUFAs having the higher nutritional value (FAO/WHO, 1994). FAO/WHO(1994) recommend a ratio of n-3/n-6 in the human diet of 0.1-0.2, and more than that is better for human health. In the groups tested in this research on *P. trituberculatus*, the Σn-3PUFA/Σn-6PUFA ratio (hepatopancreas, gonads and muscles) was greater than 0.2, indicating that the commercial feed group was inferior to the trash fish

group, especially in terms of the hepatopancreas and gonads, with little impact observed with the mixed feeding model. Whether it is possible to improve the fatty acid quality of *P. trituberculatus* by enhancing the content of DHA and HUFA in the compounded feed in the future will require further research.

In the three feeding modes, the content of amino acid in the gonads of the group fed entirely with the commercial feed was less than that observed with the mixed feeding group and the trash fish group, which may be related to the hypoplasia observed in the gonads of the crabs fed entirely with the commercial feed model. In a former study, He et al. (2018) reported that the content of amino acids in the gonads of female *P. trituberculatus* is poor at the early stage of development (lower GSI) and gradually increases with gonad development (higher GSI). However, in our research, the GSI of *P. trituberculatus* was small; hence, a lower content of amino acids is present. The difference in the amino acid content in the muscle of *P. trituberculatus* between the three diets was relatively small. Moreover, the content of EAA in the muscle of *P. trituberculatus* surpassed that of the trash fish group under complete feeding with the complex diet, On the other hand, this EAA content was attributed to the relatively high conserved nutritional composition of the *P. trituberculatus* muscle because the high content of EAA in the compounded feed, which was well absorbed and retained in the muscle. The essential amino acid fraction (EAAS) and the ratio of essential amino acid to total amino acid (EAA/TAA) is a significant indicator for evaluating the nutritional value of amino acids in aquatic products (Wu et al, 2014). The EAAS of a certain amino acid in food is greater than 100, which indicates that the amino acid is a non-restrictive amino acid. The ideal ratio of EAA/TAA is approximately 0.4 (FAO et al, 1985). Generally, the nutritive value of amino acids in the gonads of *P. trituberculatus* fed with the formula diet was the lowest value. The Ile, Leu, Lys, and Phe+Tyr values were no more than 100, which indicated they were restrictive amino acids, but the nutritive values of the amino acids in muscle were minimal because their amino acid score was highest. In contrast, the nutritional values of the amino acids in the gonads and muscles of *P. trituberculatus* were optimal in the mixed diet group, whereas the nutritional value of amino acids in the gonads and muscles of *P. trituberculatus* were optimal in the mixed diet group.

Conclusion

The results of this study show that the gonadal dysplasia of *P. trituberculatus* occurred with those crabs fed entirely with commercial formulated feed. Conventional biochemical values, fatty acid content and amino acid composition in the gonads, hepatopancreas and muscles were poor. However, the mixed feeding mode of the commercial formulated feed and trash fish had little effect on the gonad development and nutritional composition of *P. trituberculatus* but could improve the content and quality of the amino acids in the gonads and muscles. Therefore, this study concludes that the mixed feeding of commercial formulated feed and trash fish is an ideal feeding mode because it not only alleviates the dependence of *P. trituberculatus* breeding on trash fish resources but also guarantees the excellent quality of *P. trituberculatus*.

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